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Errors in Protein-testing, and their Consequences

Farmers don't grow wheat or barley - they grow money, and **Protein** is **Money** to wheat growers. Protein premiums represent much-needed additional income to wheat-growers. Accurate and reliable protein-testing is more important than grading - a protein spread of 1.0 % (e.g. 12.0 - 13.0) can be much more valuable than a change of grade 2CW to 1CWRS wheat. This article is intended to tell some truths concerning protein-testing.

Introduction

Since August 1st, the beginning of the 1999-2000 crop year, farmers and grain elevator companies who deliver the top two milling grades of Canada Western Red Spring (CWRS) have received premiums for protein content on the basis of 0.1 % increments. This system replaced the original system, whereby protein premiums were paid on the basis of 0.5 % increments.

The change will improve to farmers (in particular) the probity of receiving protein premiums. Under the previous (0.5 % increment) system, many farmers were disappointed to learn that the protein content of their wheat was 13.4, or 12.9 %, thereby missing out on the higher premiums.

But the new system will focus renewed attention onto the accuracy of protein-testing. Practically all CWRS wheat in western Canada is tested for protein content by a **N**ear-**I**nfra**R**ed (NIR) instrument, either at country delivery points, or at terminal elevators in Vancouver or Thunder Bay. The reference method used by the Canadian Grain Commission (CGC) for calibration and monitoring is the Dumas method, or Combustion Analysis. The sample is burned in oxygen, and the protein nitrogen measured directly.

Truth No. 1. There is no such thing as an absolutely error-free test for protein content.

The Dumas method replaced the Kjeldahl method, partly because it is essentially chemical-free, but mainly because it is more precise. The error of protein-testing is determined by repeated analysis of a very well-blended and sub-sampled sample of the material to be tested, in this case CWRS wheat. The error for Dumas testing is 0.09 %, whereas that of the Kjeldahl method was 0.14%. An earlier article on this web-site described the Dumas test, and cited about 12 sources of error.

Table 1 gives typical results of testing a sample of wheat with different degrees of error. This set of data was compiled to provide a mean protein content of 13.6 % (13.5 % moisture basis), which is the long-term average protein content of western Canadian CWRS wheat. Notice that at an error of 0.30 %, the range in protein content (of 12 samples) was over 1.0 %. These ranges in individual results are characteristic of the spread obtained with the errors stated.

Table 1. Typical results of protein testing with different degrees of error

Mean	13.59	13.56	13.57	13.62	13.64	13.58
Error	**0.05**	**0.10**	**0.15**	**0.20**	**0.25**	**0.30**
High	13.5	13.5	13.7	13.8	13.9	14.1
Low	13.3	13.2	13.1	13.2	13.1	13.0

There is more to this than meets the eye. Unfortunately we can't escape mathematics! Mathematically only about 67 % of all tests on the same sample will lie between plus or minus the error from the mean. In this example in 100 tests on the same sample, an error of 0.15 would place 67 % of the results between 13.6 + 0.15 (13.75) and 13.6 - 0.15 (13.45), which rounds off to a range of 13.8 to 13.4 %.

It also means that the other 33 % of the results will be **outside** this range. About 95 % of all results would be within twice the error from the mean, and 98 % of the results would fall within 3 X the error from the mean. Table 2 gives the range you can expect for the results of testing the same sample 100 times with the five levels of error in Table 1.

Table 2. Ranges in protein results at different error levels

Error level		0.05	0.10	0.15	0.20	0.25	0.30
Mean protein content = 13.6 %							
1 X Error	High	13.65	13.7	13.75	13.8	13.85	13.9
67 % of	Low	13.55	13.5	13.45	13.4	13.35	13.3
results	+/-	0.05	0.10	0.15	0.20	0.25	0.30
	Range	0.1	0.2	0.3	0.4	0.5	0.60
$ value per tonne		**0.66**	**1.43**	**1.98**	**2.64**	**3.30**	**3.94**
2 X Error	High	13.7	13.8	13.9	14.0	14.1	14.2
95 % of	Low	13.5	13.4	13.3	13.2	13.1	13.0
results	+/-	0.10	0.20	0.30	0.40	0.50	0.60
	Range	0.2	0.4	0.6	0.8	1.0	1.2
$ value per tonne		**1.32**	**2.64**	**3.97**	**5.29**	**6.61**	**7.88**
3 X Error	High	13.75	13.9	14.05	14.2	14.35	14.5
98 % of	Low	13.45	13.3	13.15	13.0	12.85	12.7
results	+/-	0.15	0.30	0.45	0.60	0.75	0.90
	Range	0.3	0.6	0.9	1.2	1.5	1.8
$ value per tonne		**1.98**	**3.97**	**5.95**	**7.94**	**9.92**	**11.89**

The "dollar value per tonne" figures are based on an arbitrary spread of about $42.00 from 11.9 to 15.0 % protein, and give the value of a positive or negative

difference from the "true" protein result. These values can be expected to vary considerably, depending on the availability of protein. For example, premiums are higher in low protein years when protein is in short supply, and lower in high protein years. A grade differential between 1 CWRS and 2 CWRS usually means only about $3 - 4.00 per tonne.

These results are the "Real World" - this is what is bound to happen if a sample of wheat is tested 100 times with these different levels of error. The other 2 results (100-98) will be even further away from the mean. Unfortunately the laws of mathematics are **absolute** - so if 2 results of greater than +/- 0.75 from the mean do not show up in the first 100, there will be 4 such results in the next 100 tests, and so on.

Truth No. 2. A positive or negative difference from the "true" result means that clients can gain, as well as lose, on a protein test.

What are the levels of error of protein-testing in western Canada?

Since 1981 the Grain Research Laboratory (GRL) of the CGC has provided the Elevator companies of western Canada with a free check sample service for both CWRS and CWAD wheat classes. The purpose is to help operators of company NIR instruments to determine how accurate and consistent their instruments are, relative to the reference method. About 10 Kg of CWRS or CWAD wheat are thoroughly blended, by GRL staff. Duplicate sub-samples are taken, ground on a U-D Cyclone grinder, well-blended, and tested for protein and moisture content by reference methods (the Dumas method for protein, and the 130^{0} C oven method for moisture contents). The results are reported on the constant moisture basis of 13.5 %. The bulk sample is divided into 300g sub-samples, which are sent out to the head offices of the western Canadian Elevator companies. Extra samples are sent to companies on request. This free service has been in place for nearly 20 years.

A report accompanies the sample, with the results of the previous month's sample. The protein content of the present sample appears on the envelope, as a guideline to the operator. Elevator companies are coded, so that each can recognize their own results. Over 300 sets of results are submitted to the GRL every month. The average error for most of the companies is 0.150 %. In a typical month the range between highest and lowest results has been 0.52 %. This has varied between 0.3 and 0.8 %. These tests are presumably carried out by the most competent operators in each company. The error of the Dumas test, the reference test for protein content at the GRL is 0.1 (actually 0.09) %. This is very strictly monitored.

Truth No. 3. Sampling and sample preparation are the most important sources of error .

This is true, no matter what test method is used, and a future article on this web-site will discuss sampling methods.

Sampling itself is responsible for a third of the total error in protein-testing. The sample has to be representative of the delivery. This can be achieved at the farm by taking a handful of wheat every few minutes during the whole time the truck is being loaded. The handfuls should be put into a pail, then when the truck is finished loading the contents of the pail must be very thoroughly mixed, and a bag-full taken as the sample to be used as a reference for protein-testing. During harvest-time this method is not practicable, since farm trucks may be loaded directly from the combine, sometimes while the combine is still moving. The sample can also be drawn from the truck after loading, by using a sampling probe at at least six locations covering the full depth of grain in the truck. These six sub-samples must then be thoroughly blended, as before.

At the elevator the elevator staff should sample the truck continuously during unloading. A good sample can be taken by diverting a handful every half-minute or so into a pail, or by probing the truck, as mentioned above. The sample should be very well mixed before carrying out the protein test. Cleaning the sample may not be sufficient to give a good blend, and the sample should be well-mixed *after* cleaning if it is to be used for a protein test.

Truth No. 4. Precision is more important than accuracy.

Accuracy means closeness to the true result (in this case the reference result). **Precision** means the degree to which the same result can be obtained by repeated tests of the same sample.

Accuracy can be changed in most NIR instruments by changing the intercept of the calibration equation. So the actual Accuracy of the instrument can be set so that it reads the true (reference) result, or a higher or lower value than the true result.

Precision is a measure of the instrument performance, and can not be changed in the same way. It is affected by both the instrument itself (including the calibration), and the operator. Only once is the instrument calibrated to provide accurate results, but the precision affects the instrument performance every time it is used. It gives a measure of the ability of the instrument to produce the same result for the same sample consistently.

Accuracy and precision should be checked every day, the accuracy by comparing the results with those of certified reference values, and the precision by determination of the variability of the instrument in testing the same samples over a period of at least a few days. The monthly check sample service supplied by the CGC/GRL enables the operators to do both of these tests. The mean result of several tests gives a measure of the accuracy, and the variability from day to day gives the precision. The results of both are sent to the elevator companies each

Truth No. 5. Grinding is a major source of error.

The older NIR instruments require a ground sample, which is loaded into a test cell for reading on the instrument. Both grinding and loading the cell are important sources of error. Table 3 shows the influence of grinding the sample on the error of reference testing. Single tests carried out on separately ground samples gave much higher errors than duplicate tests carried out on the same ground samples. The table also shows the influence of grinding and cell-loading on NIR tests carried out on instruments such as the Technicon (now Bran+Luebbe) InfraAlyzer, DICKEY-john InstaLab, and Perten Inframatic instruments. Both the grinding and cell-loading errors were significant. The "Single cell-loading precision" is the error of the instrument itself, with no operator error.

Table 3. Influence of grinding and cell-loading on precision of Reference and NIR testing of wheat

Cell-loading procedure	Reference		Near-infrared	
	Protein	Moisture	Protein	Moisture
Fresh grind precision (Error)	0.18	0.27	0.27	0.37
Single grind precision*	0.10	0.07	0.14	0.12
Single cell-loading precision	-	-	0.06	0.05

* Ground sample re-tested.
** Ground sample re-tested without re-loading sample cell (NIR only).

The error was much lower (precision was better) when the NIR instrument was used to test the sample without re-grinding or re-loading, which showed that the instrument itself was very precise, and most of the error came from the operator. Whole-grain analyzers, such as the Tecator InfraTec and Perten Model 9100 instruments, eliminate both the grinding and cell-loading errors in NIR protein-testing, and are recommended for the best results.

Truth No. 6. Foreign material is a significant source of error in whole-grain NIR analyzers.

Materials such as small seeds (weed seeds or canola seed) or chaff and pieces of straw change the way in which the light passes through the sample, and cause more variability in the results of testing wheat. A NIR protein test should be carried out *after* the sample has been cleaned and re-blended.

What does all this really mean?

1. There is no test for protein content that is completely free of errors, and errors in protein-testing are bound to happen. It is in everyone's interest to keep them low.
2. Because of this, if a farmer takes a sample to 2 or more different elevators it is **very likely** that the results will not agree with each other **exactly**.
3. Errors work both ways, and when a sample is tested for a farmer or an elevator manager (at primary elevators) the error is just as likely to be on the high, as on the low side.
4. Errors mean money - but again they work both ways, and with payments being made on the basis of 0.1 % increments a farmer is just as likely to gain a little as to lose a little as a result of the errors.
5. Careful sampling, cleaning the sample (even roughly removing most of the dockage and small seeds) and careful testing will reduce the errors.
6. In view of the monetary importance of protein both farmers and elevator managers should be able to appeal a protein result for a small fee, by sending samples into a Canadian Grain Commission Service Centre for verification in the same way as samples are submitted "Subject to grade and dockage". The test will be done by an experienced operator, using a NIT instrument that is monitored for accuracy from the Industry Services Head Office in Winnipeg.

Since the normal error of the test is about 0.2 %, adjustment in payment should only be made if the CGC result differs from the original result by more than 0.4 %

So - what should be done to ensure the most efficient protein-testing on the Prairies?

Three groups of people are involved - the farmers themselves, elevator managers and their head offices, and the CGC:

1. Farmers can help by making sure that they:

a. take the most representative samples they can from the wheat they intend to deliver for protein premiums during loading their trucks. They could also take samples of wheat in storage bins, by taking a handful periodically from the back of the truck during filling the bins via the auger. All samples should be thoroughly blended before testing. This can be done by hand.

b. store the sample in plastic bags, preferably double plastic bags, to make sure that the moisture doesn't change between taking the sample and doing the protein test, and

c. store the bag of sample carefully between taking the sample and getting it tested, and not allowing it to get too hot or cold. Room temperature in the house will be fine, but don't, for example, put it on the floor of the truck near the heater.

2. Elevator managers can help by:

a. Taking the most representative sample they can from trucks during unloading at the elevator

b. Cleaning the sample, and blending it thoroughly before testing

c. Checking their NIR protein tester every day to make sure that it is giving the right results for a check sample or samples

d. Making sure that the sample submitted from the farmer is close to the room temperature before testing

3. Elevator company head offices can help by:

a. Continuing to participate in the CGC/GRL Check Sample service

b. Making sure that their protein testers are giving accurate and precise results according to the reference results of the CGC/GRL samples

c. Preparing their own check samples and sending them out to their delivery points for use by elevator managers - this would involve preparation of probably as much as 200 Kg of each sample for some companies, and they would require equipment to do the all-important blending and accurate sub-dividing

d. Ideally setting up their own networking system, whereby the instruments at delivery points are monitored and controlled from head office. Alternatively the company could obtain this service from elsewhere on a contract basis.

4. The Canadian Grain Commission can help by:

a. Continuing to ensure the highest degree of accuracy in their reference testing by Dumas (Combustion Analysis)

b. Continuing to provide the Check Sample service. Due to the requirement for cost recovery by the CGC, a small fee may be initiated on or after August 1, 1999, to cover labour of sample preparation, mailing and data analysis. Elevator companies would likely be billed annually for this service

c. Providing samples of certified protein results to companies who provide monitoring and control service to elevator companies

d. Certifying instruments as acceptable for protein-testing of wheat

e. By introducing a "Subject to Inspector's protein result" system, similar to the present "Subject to grade and dockage" appeal system, whereby samples can be submitted for re-testing by the CGC for a modest fee.

Accurate protein-testing is possible, but it will take time - maybe a year or more - before all of these steps become implemented. But as they are implemented the overall reliability of protein-testing on the Prairies will gradually improve.

Protein is a very important subject, and one which has always been taken seriously by the Canadian Grain Commission. Several questions are repeatedly posed to the CGC. These include:

Question No. 1. Why does protein change in the bin?
Question No. 2. Why do protein testing results vary so much even when testing the same sample?
Question No. 3. Why doesn't the CGC grade on the basis of protein?

A future article in planned for this web-site to answer these, and other on-going

questions.

For more information available on this site

Protein Testing Methods at the Canadian Grain Commission by Phil Williams, Debbie Sobering and John Antoniszyn

Commission Facts. Taking a Representative Sample

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